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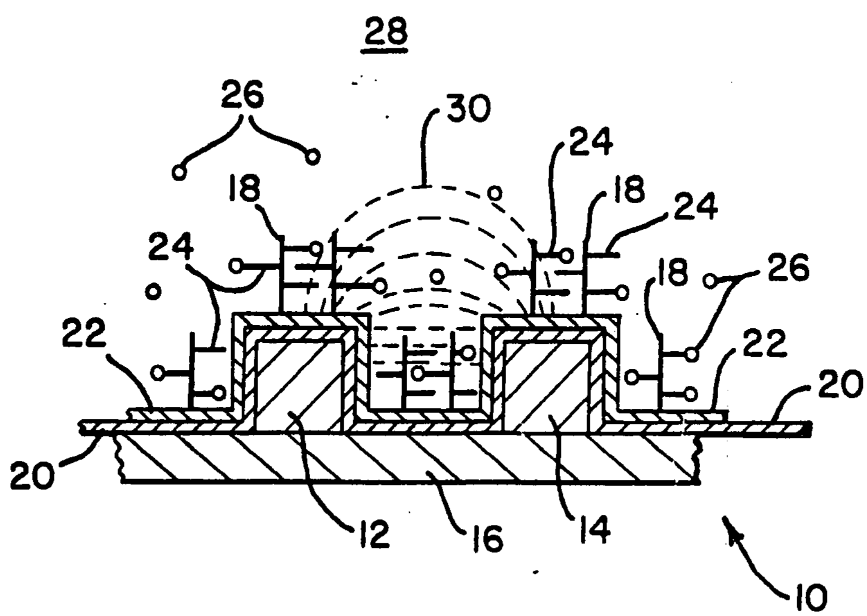
(54) Title: THREE DIMENSIONAL BINDING SITE ARRAY FOR INTERFERING WITH AN ELECTRICAL FIELD



(57) Abstract

A dielectric material of a capacitive affinity sensor (10) has a three-dimensional molecular binding site array. A glass base (16) is layered with a binding agent (22) like silane from which a polymeric backbone (18) like polylysine extends. The polymeric backbone (18) is prepared to accept receptor molecules (24) like cortisol hemisuccinate to bind a specific antibody (26). Such an array changes dielectric properties between the two electrodes (12, 14) of the capacitive affinity sensor (10) to greatly enhance sensitivity of the sensor (10).

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THREE DIMENSIONAL BINDING SITE ARRAY FOR INTERFERING WITH AN ELECTRICAL FIELD

Background of the Invention

Cross-reference is made to two U.S. Patent Applications: Serial Number 044,767, for Added Array Of Molecular Chains For Interfering With Electric Fields, by W.D. Stanbro et al.; and Serial Number 044,769, for Sintered Pellet With Biochemically Active Layer, by A.L. Newman, which were filed the same date and were assigned to the same entity as this application.

The invention relates to a means for interfering with an electrical field. More specifically, the invention relates to electrodes of a capacitive affinity sensor that are insulated with a three-dimensional molecular binding site array.

In composition analysis, capacitive sensors have been used to determine the concentration of a specific gas in a mixture, or an analyte in a fluid, for example. Such sensors measure a capacitance that changes with the concentration.

Newman U.S. Patent Application Serial No. 799,761, filed November 19, 1985, ("the Newman Patent Application") involves a capacitor for determining the concentration of an analyte in a fluid, for instance. Biospecific binding reactions occur in a space between electrodes of a capacitive sensor. These reactions occur among molecules of a binding agent immobilized on a surface and an analyte in a fluid. These reactions result in the displacement of small fluid molecules having high dielectric constants by large biochemical molecules having low-dielectric constants. This displacement of molecules changes the dielectric constant of the capacitor.

Raymond et al. U.S. Patent 4,571,543 discusses a capacitor for detecting and measuring the concentration of specific non-aqueous materials or constituents in fluids. The capacitor is layered with a coating of silane and then

a coating of certain polymers. These polymers form membranes that are permeable to constituents of the fluids. The constituents penetrate through the membrane to change the dielectric constant of a solution under the membrane.

Volgyesi U.S. Patent 4,453,126 concerns a capacitor for monitoring the concentration of anaesthetic gas in a breathing mixture. The capacitor has a dielectric of lipids or elastomers which permit the absorption of the anaesthetic gas to vary electrical characteristics of the sensor.

Arwin et al. U.S. Patent 4,072,576 relates to a capacitor for studying enzymatic activity and for studying an immunological binding reaction. A polypeptide substrate is used to study enzymatic activity and an antigen is adsorbed onto an electrode surface to study the binding reaction of the antigen to a biospecific antibody.

Molecular Design for Electroanalysis, by Murray et al., Analytical Chemistry, Vol. 59, No. 5, March 1, 1987, discusses chemically modified electrodes for use in sample analysis, and the use of electroactive polymer films, like poly-L-lysine, on such electrodes. These films facilitate oxidation-reduction reactions at the electrodes.

Kinetics of Electron-Transfer Cross-Reactions within Redox Polymers; Coatings of a Protonated Polylysine Copolymer with Incorporated Electroactive Anions, by Anson et al., Journal of the American Chemical Society, Vol. 105, No. 15, 1983, p. 4884, describes electrodes coated with polymer layers that form a three dimensional arrangement of catalytic sites. These layers comprise a random orientation of polymer coils to facilitate oxidation-reduction reactions at the electrode. New Model for the Interior of Polyelectrolyte Coatings on Electrode Surfaces; Mechanisms of Charge Transport through Protonated Poly(L-lysine) Films Containing $Fe^{III}(edta)^-$

and $Fe^{II}(edta)^{2-}$ as Counterions, by Anson et al., Journal of the American Chemical Society, Vol. 105, No. 5, 1983, p. 1096, also describes such electrodes.

In composition analysis, affinity chromotography has been used to determine the presence or concentration of an analyte in a fluid. The analyte is chemically separated or isolated from the fluid, as described in two articles entitled "Affinity Chromotography", one by I. Parikh et al., August 26, 1985, Chemical and Engineering News, pp. 17-32 and the other by R. Walters, September, 1985, Analytical Chemistry, Volume 57, No. 11, pp. 1099A-1114A.

Maggio et al. U.S. Patent 4,233,402 deals with the chemical analysis of an analyte on a substrate using hub nuclei that spread along the substrate and to which ligands are covalently bound.

Summary of the Invention

The invention concerns an apparatus, and a method for making the apparatus, comprising a base layer, an electrical field generating means on the base layer, and an electrical field interfering means. The electrical field interfering means has a polymer backbone having a means for accepting a receptor molecule. A means is provided to bind the polymer backbone to and extend the polymer backbone from the base layer. In one embodiment, the electrical field generating means is an electrode of a capacitive affinity sensor, for instance.

In that embodiment, a biochemical layer is provided between electrodes of the sensor. The biochemical layer has greatly decreased dielectric properties and a greatly increased thickness. Thus, the capacitance of the biochemical layer is greatly decreased. Such a biochemical layer is used to provide a very sensitive capactive affinity sensor, for instance.

Brief Description of the Figures

Figure 1 shows electrodes of a capacitive affinity sensor with a dielectric material according to this invention.

Figure 2 shows schematically an electrode insulated with a structure having a three dimensional array of molecular binding sites.

Figure 3 shows one composition of the three dimensional array of Figure 2.

Detailed Description

Capacitive affinity sensors measure the concentration of an analyte by detecting a change in capacitance as an analyte molecule moves in or out of an electric field between two electrodes of the sensor, for instance. The moving analyte molecule has a low dielectric constant and displaces solvent molecules having higher dielectric constants from a biochemically active layer between the two electrodes. The displacement of the solvent molecules by the analyte molecules reduces capacitance between the two electrodes. The capacitance between the two electrodes is inversely proportional to the concentration of the analyte being measured by such a sensor.

Other capacitances are present in a capacitive affinity sensor and include the capacitance of any passivating layers over the electrodes and the capacitance of the solvent about the electrodes. The capacitances of the sensor add as follows:

$$C_T = \frac{1}{\sum_{i=1}^{n} \frac{1}{C_i}} \tag{1}$$

where C_T is the total capacitance of the sensor and C_i is the capcitance of each of the biochemically active layer, the passivating layer and of the solvent. For an idealized parallel plate capacitor, the individual capacitances are proportional to the ratio of the

dielectric constant and the distance between the parallel plates. That is

$$C_i \propto \frac{\epsilon_i}{d_i} \qquad (2)$$

where ϵ_i is the dielectric constant and d_i is the distance between the parallel plates. This type of situation applies regardless of the actual geometry of the plates.

A typical passivating layer of a capacitive affinity sensor is about 2000 Angstroms thick and provides an impervious pin-hole free barrier to water and ions. A solvent layer can be several microns thick. However, in a sensor as discussed in the Newman Patent Application, antibodies extend about 100 Angstroms above an insulator surface in the biochemically active layer. Thus, such a biochemically active layer is thin compared to the passivating layer and the solvent layer. According to equation (2) the capacitance of such a biochemically active layer is large compared to that of any passivating layers and solvent. According to equation (1), the dominant capacitance in the total capacitance C_T is that of the layer having the lowest capacitances. Thus, it is desirable to minimize the capacitance of the layer of an affinity sensor that is modulated, such as the biochemically active layer, to maximize the sensitivity of such a sensor. Minimizing the capacitance of the biochemically active layer brings the capacitance of this layer into the ranges of the other capacitances in the sensor.

The capacitance of the biochemically active layer is affected by the presence or absence of the large analyte molecules in the solvent, for instance. According to equation (2), capacitance decreases with decreasing ϵ_i or increasing d_i. This invention concerns a means for increasing d_i. This invention also concerns a means for increasing the difference between capacitances that are

measured when all the large molecules enter the electric field and when all leave the electric field.

Figure 1 shows schematically a capacitive affinity sensor 10 with electrodes 12 and 14 insulated according to this invention. The sensor 10 has a base layer 16 that supports the two electrodes 12 and 14, which have opposite polarities. The base layer 16 comprises a substrate of insulating material like alumina.

A passivating layer 20 covers the base layer 16 and electrodes 12 and 14 in the preferred embodiment of this invention. A binding agent 22 covers the passivating layer 20. This binding agent 22 binds polymeric backbones 18 to the base layer 16. These polymeric backbones 18 extend roughly at right angles to any surface covered by the binding agent 22. There are a number of potential materials for the backbone including polynucleotides, polysaccharides, polystyrenes and polypeptides. Though not shown, horizontal polymeric backbones extend from any vertical surfaces covered by the binding agent 22.

Molecules form receptors 24 that branch from the polymeric backbone 18. Each receptor 24 is a potential binding site for a molecule of a specific analyte 26. The receptors 24 are located, not only in a plane parallel to the surface of the binding agent 22, but at different distances above the binding agent 22. The receptors 24 are located at different heights of the polymeric backbone 18. Thus, a three dimensional array of receptors 24 is provided for molecules of the analyte 26.

This array of receptors 24 allows a greater number of analyte molecules 26 to bind in the electric field 30 between the electrodes 12 and 14, compared to the number of analyte molecules 26 that could bind to receptors located only along the surface of the binding agent 22. Also, this greater number of analyte molecules 26 bind and then displace a greater amount of high-dielectric constant solvent 28 from the electric field 30.

The use of a three dimensional array of receptors 24 greatly increases the thickness d_i and greatly changes the dielectric properties of a biochemical layer in a capacitive affinity sensor. According to equation (1), the capacitance between electrodes 12 and 14 will greatly decrease.

Figure 2 shows schematically a detail of the three dimensional array of receptors 24. The binding agent 22 covers the base layer 16 and binds the polymeric backbones 18 to the base layer 16. Receptors 24 are located along the polymeric backbones 18 at different distances from the binding agent 22 and the base layer 16. Large molecules 26 of an analyte travel through the solvent 28 and bind to the receptors 24. The receptors 24 are biospecific to the large molecules 26 of the analyte, for instance.

The base layer 16, polymeric backbones 18, binding agent 22, receptors 24 are adjacent to, and in the electrical field of, an electrode 12 of a capacitive affinity sensor as shown in Figure 1, for instance. These elements 16, 18, 22, 24 and 28 in this electric field form an electrical insulator 32 for a single electrode 12 as shown in Figure 2. However, in the preferred version, the base layer 16, polymeric backbones 18, binding agent 22, receptors 24, and solvent 28 form a dielectric material between two electrodes of a capacitive affinity sensor.

As discussed above concerning the affinity sensor 10 of Figure 1, the large analyte molecules 26 have low dielectric constants and displace the solvent molecules 28 having a high dielectric constant as the analyte molecules 26 bind to the receptors 24. Thus, the insulating qualities of the insulator 32 vary proportionally with the concentration of analyte molecules 26 in the solvent 28.

Figure 2 shows the insulator 32 adjacent one electrode 12. The relative arrangement of the insulator 32 and electrode 12 is easily varied. For instance, a passivating layer 20 and the binding agent 22 may cover

both the electrode 12 and the base layer 16 as shown in Figure 1. The passivating layer 20 and binding agent 22 may cover only the base layer 16, in which case the binding agent 22 may contact the electrode 12 as shown in Figure 2 or may be spaced from the electrode 12. The insulator 32 is adjacent the electrode 12 when both are arranged so the insulator 32 substantially interfers with the electric field of the electrode 12.

Figure 3 shows one composition for the insulator 32 of Figure 2. The first step in forming this composition is the preparation of a silanized surface. The base layer 16 is glass-like such as silica with an SiOH layer. The base layer 16 is dipped in a 2% solution of 3-aminopropyltriethoxy silane in 95% ethanol for 1 to 2 minutes. This forms the silanized surface which is set aside for a number of hours to cure. Other silanes may be used such as:

phenyltriethoxysilane,
chloropropyltriethoxysilane,
vinyltriethoxysilane,
allyltriethoxysilane, and
cyanoethyltriethoxysilane.

The next step is the preparation of polylysine backbone protected amino groups. Amino groups of the polylysine backbone are to be protected with N-carbobenzoxy ("CBZ") on the α nitrogen 36 and the ϵ-nitrogen 38 to prevent cross binding of the polylysine to itself, instead of only to the silane binding agent 22. The α nitrogen 36 can be protected by treating the polylysine with CBZ chloride. Polylysine with CBZ protected ϵ nitrogens 38 is commercially available from Sigma Chemical Company. Some examples are:

poly- -CBZ-D-lysine, No. P6256
poly- -CBZ-DL-lysine, No. P2883

poly- -CBZ-L-lysine, No. P4510 (1000-4000 molecular weight) and

poly- -CBZ-L-lysine, and P9503 (200000-500000 molecular weight).

Next, the polylysine with protected nitrogens 36 and 38 is connected to the silane covered base layer of the sensor. The sensor is immersed in phosphate buffered saline having a pH of 6.4, with 1-ethyl-3-(3)-dimethylaminipropyl (carbodiimide), known as EDC, and the polylysine is then added. The polylysine binds to the silane binding agent and forms a backbone 18 substantially perpendicular to the base layer 16. The CBZ is then removed by catalytic hydrogenation.

The final step is the addition of a linking molecule with a biospecific receptor 24, to the ε nitrogens 38 of the polylysine backbone 18. In this final step, the sensor is immersed again in phosphate buffered saline with EDC to which cortisol hemisuccinate is added, for instance. The hemisuccinate attaches as one example of the linking molecule and the cortisol as one example of the biospecific receptor 24. Alternately, the cortisol could be attached directly to the polypeptide without a linking molecule. With or without a linking molecule, the biospecific receptor 24 is chosen to bind with a specific analyte.

Examples of biospecific receptors are discussed in the Newman Patent Application. The following chart lists other examples of receptors and analytes that are biospecific to and, therefore, bind to one another.

Receptors	Analytes
Antigen	Antibody
Hapten	Antibody
Enzyme	Substrate Chemical
Lectin	Carbohydrate
Hormone	Hormone Receptor
Hormone	Binding Globulin

Neuroreceptor	Neurotransmitter
DNA	RNA
DNA	DNA
RNA	RNA

These receptors and analytes are reversible. For instance, when an antibody is bound as a biochemcially active layer, a biospecific antigen diffuses through a solvent to bind onto that antibody.

A polypeptide backbone with a cortisol receptor is ideal for a capacitive affinity sensor using competitive binding. A capacitive affinity sensor using competitive binding is described in the Newman Patent Application. This patent also describes a sensor using direct binding.

According to the present invention, a capacitive affinity sensor using direct binding has a cortisol receptor 24 that attaches to the polypeptide backbone. The cortisol receptor will bind with an analyte of cortisol binding globulin.

A polysaccharide may also be used as the polymeric backbone. An example of such a polysaccharide is cyanogen bromide activated agarose. This agarose would bind with a 3-aminopropylsilane group on the surface of a base layer, using EDC as a catalyst in a solvent like water or alcohol. The agarose is then reacted with a linking molecule such as 1, 4-diaminobutane, again using cyanogen bromide to activate the agarose and using EDC as the catalyst.

A polynulceotide may be used as the polymeric backbone. An example of such a polynucleotide is polyadenylic acid, which is commercially available from Sigma Chemical Company, No. P9403. Amino groups of the andenylic acid units are protected by attaching acetyl groups to them. Then the terminal phosphoryl groups of the andenylic acid units are attached to an amino silane on the base layer of a sensor using EDC as a catalyst. The protecting acetyl groups are then removed from the

amino groups by treating the sensor with ammonium hydroxide. Finally, the cortisol hemisuccinate would be attached to the adenylic acid amino groups using EDC as a catalyst.

According to a preferred version of this invention, polymeric backbones form a biochemically active layer comprising a three dimensional binding site array for analyte molecules. This array thickens a dielectric material of a capacitive affinity sensor and drastically affects the dielectric properties of the sensor. The array enhances the sensitivity of the sensor to an analyte in a solution, for example.

What is claimed is:

1. An apparatus comprising:

a base;

a means on the base for generating an electrical field;

a means for interfering with the electrical field comprising a polymeric backbone having a means for accepting a receptor molecule; and

a means for binding the polymer backbone to and for extending the polymer backbone from the base.

2. The apparatus of claim 1, the means for binding comprising a silane, and the means for generating an electric field comprising an electrode.

3. The apparatus of claim 2, the polymeric backbone comprising a polylysine.

4. The apparatus of claim , the polymeric backbone comprising a polysaccharide.

5. The apparatus of claim 2, the polymeric backbone comprising a polynucleotide.

6. The apparatus of claim 2 comprising a capacitive affinity sensor, the means for generating an electric field comprising two electrodes having opposite polarities, and the means for interfering comprising a dielectric material between the two electrodes.

7. The apparatus of claim 6, the polymeric backbone comprising a polylysine.

8. The apparatus of claim 6, the polymeric backbone comprising a polysaccharide.

9. The apparatus of claim 6, the polymeric backbone comprising a polynucleotide.

10. A method comprising:

positioning a means for generating an electrical field on a base;

layering a binding agent on the base adjacent the electrical field generating means;

extending a polymeric backbone from the binding agent; and

preparing the polymeric backbone to accept a receptor molecule.

11. The method of claim 10 comprising making the polymeric backbone by protecting amino groups of a polymer with a protecting molecule.

12. The method of claim 11 comprising removing the protecting molecule.

13. The method of claim 12 comprising adding a receptor molecule to the polymeric backbone.

14. The method of claim 13 comprising forming a polypeptide backbone.

15. The method of claim 13 comprising forming a polynucleotide backbone.

16. The method of claim 13 comprising forming a polysaccharide backbone.

17. The method of claim 13 comprising positioning two electrodes on the base of a capacitive affinity sensor, and layering the binding agent between the two electrodes.

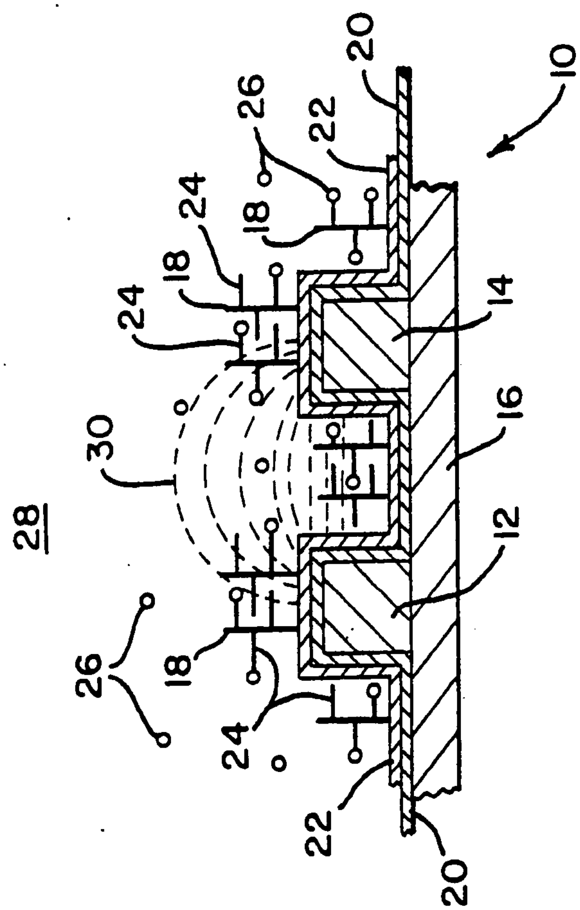
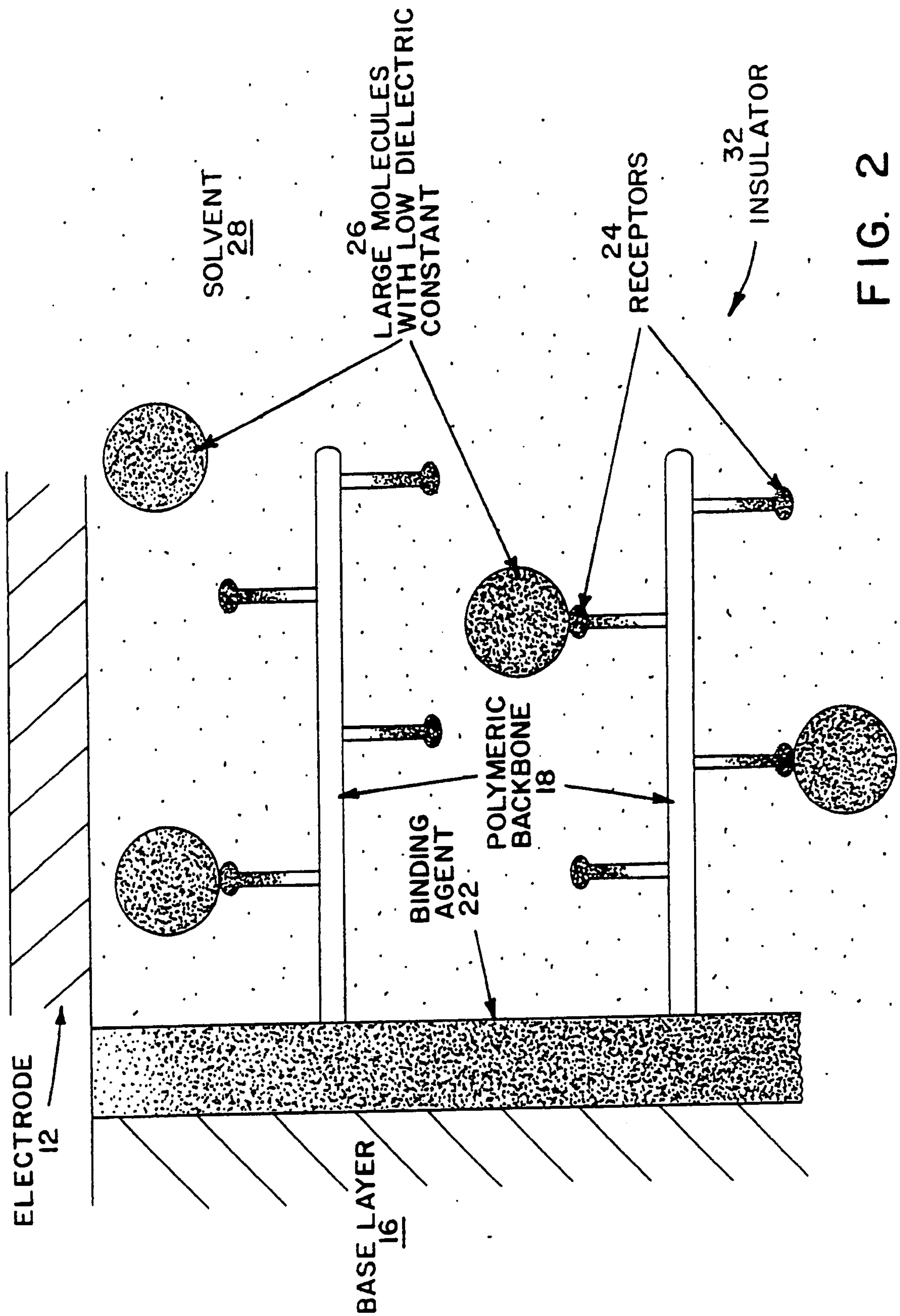


FIG. 1



SOLVENT 28
26 LARGE MOLECULES WITH LOW DIELECTRIC CONSTANT
24 RECEPTORS
32 INSULATOR
POLYMERIC BACKBONE 18
BINDING AGENT 22
ELECTRODE 12
BASE LAYER 16

FIG. 2

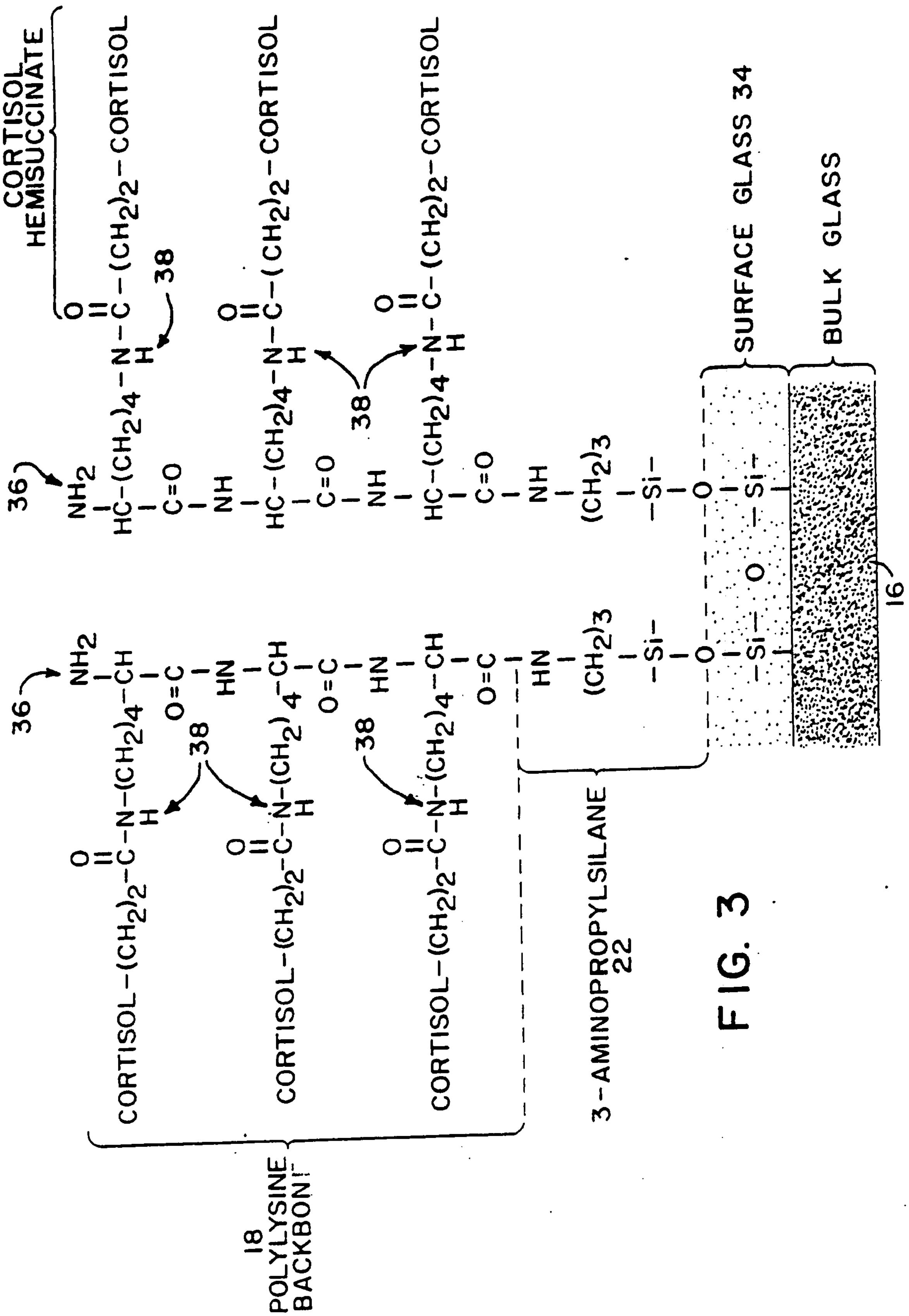

CORTISOL HEMISUCCINATE
SURFACE GLASS 34
BULK GLASS
16
36
38
3-AMINOPROPYLSILANE 22
18 POLYLYSINE BACKBONE

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/01431

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): G01R 27/26; G01N 27/22
US. Cl.: 422/68; 324/61R: 427/79

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	204/403; 324/60R,61R,61C; 422/68,70,69,90,98; 427/2,79,80,81; 435/288,291,817; 436/525,528, DIG 806

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4490216 (McCONNELL) Published 25 December 1984. See columns 42-53; Column 6 lines 3-12, 25-36; Column 7 lines 41-50; Column 8 lines 26-33, 55-60.	1,2,4-6 8-10
Y	US, A, 4637861 (KRULL) Published 20 January 1987	1,2,10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 August 1988	08 SEP 1988
International Searching Authority	**Signature of Authorized Officer**
ISA/US	MICHAEL S. MARCUS

International Application No. PCT/US88/01431

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons.

1. ☐ Claim numbers , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers___________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

Claims 1-9 are drawn to a capacitor; Class 422 subclass 68.
Claims 10-17 drawn to a method of coating; Class 427 subclass 79

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.